J. Braz. Chem. Soc., Vol. 11, No. 2, 164-169, 2000.
Printed in Brazil

0103 - 5053 $6.00+0.00

Article

Criterious Preparation and Characterization of Earthworm-composts in View of Animal Waste Recycling. Part I. Correlation Between Chemical, Thermal and FTIR Spectroscopic Analyses of Four Humic Acids from Earthworm-composted Animal Manure

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Ácidos húmicos (HA) foram extraídos de estercos de ovelhas (SH), bovinos (CO), caprinos (GO) e coelhos (RA) previamente submetidos à compostagem com a minhoca *Eisenia foetida*. As curvas de DSC dos HA mostraram eventos térmicos bem definidos indicativos de ligações intramoleculares (300 °C) e ligações organo-minerais intermoleculares (500 °C). As amostras apresentaram altos teores de nitrogênio, (de cadeias peptídicas e provavelmente N-heterocíclicos), estruturas com caráter aromático e/ou alifático insaturada com conjugação, e baixos teores de funções carboxílicas. O SH-HA foi o mais semelhante a um HA médio de solo e o RA-HA o menos semelhante. Os resultados indicam a possibilidade de se preparar vermicompostos especiais para a correção de problemas específicos de solos degradados.

Humic acids (HA) were extracted from sheep (SH), cow (CO), goat (GO) and rabbit (RA) manures which were vermicomposted with the earthworm *Eisenia foetida*. HA DSC curves showed well-defined thermal events indicative of intramolecular bonds (300 °C) and intermolecular organo-mineral linkages (500 °C). All samples showed high nitrogen contents (from peptide chains and probably N-heterocycles) and aromatic and/or unsaturated aliphatic conjugated structures, as well as a low carboxylic functionality content. The SH-HA was the most similar to a medium soil HA and the RA-HA being the least similar. The results show that it is possible to prepare distinct worm-composts to solve specific problems of degraded soils.

Keywords: humic acids, vermicomposts, thermal analysis, acid groups of humic substances

Introduction

In the South of Brazil, mainly in the southwest of the state of Paraná and the west of the state of Santa Catarina, there are serious environmental problems of soil and groundwater contamination caused by animal manure and other wastes from food industries. On the other hand, large areas of inadequately used agricultural soils are becoming arid.

The addition of humic substances (HS) to soils is known to be a remedial procedure for soil recuperation. HS enhance the soil structure through binding with clay minerals and metal oxides and modifying the equilibria of chemical reactions, which exercise an overall control on the concentration of soluble ionic species and heavy-metal traces[1]. Their environmental importance is also due to a multiplicity of other properties, for example complexing and slowly releasing metallic ions nutrients for plants and adsorbing small organic toxic compounds, such as herbicides[2,3].

The presence of HA in earthworm-composted materials has been widely demonstrated on the basis of the vermicompost (VC) fraction isolated by the conventional alkaline extraction/acid precipitation method[4]. The vermicomposting process is the result of the combined action of earthworms and microflora living in earthworms'

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Dedicated to our friend
Prof. Dr. Eduardo Stadler - DQ/UFSC (in memorian)

intestines and in the growth medium[4,5]. Earthworms accelerate manure composting by bioturbation and aeration, and also yield final products enhanced in available metallic ion nutrients for plants.

The objective of the present study was to characterize HA extracted from VC, through the correlation of chemical, thermal and spectroscopic analyses, with the aim of indicating the best way to transform the initial manure into useful materials. Knowledge of the characteristics of HA and their respective VC, from specific origins, can be very useful in proposing their use in the regeneration processes of degraded soils.

Experimental

VC were prepared from cow, sheep, goat and rabbit manure's with the earthworm *Eisenia foetida* in the biology laboratory of the CEPPA/FUNPAR/UFPR. Typically, 1.5 kg of manure and 0.15 kg of the earthworm were leaving in an open glass cylindrical flask, 20 cm of high and 4 L of capacity, pH ~ 6, in a dark room with temperature of 20 ± 2 °C and relative humidity of 55 to 75 %, during three months. HA were extracted, using a common method[6]. Briefly, the air-dried VC was treated with aqueous 0.1 mol L^{-1} NaOH under argon atmosphere for 24 h at room temperature (~25 °C). The alkaline extract was acidified with 2 mol L^{-1} HCl to pH 2 and allowed to stand for 24 h at room temperature. The coagulate (HA) was separated from the supernatant by centrifugation. In order to prevent loss or modification of important organic constituents of the samples, or excessive hydrolysis of metallic ions, the extraction of HA was carried out only once, and no treatment of the sample with an HCl/HF mixture to minimize the ash contents was carried out. This could be the reason for the relatively high ash contents of the samples. FTIR spectra were taken using KBr pellets (1 mg sample and 99 mg of spectrometry grade KBr), and recorded on a Bomen FTIR model MB100 spectrophotometer. The electronic spectra were recorded on a Hewlett Packard model 8452A-diode array spectrophotometer. The E_4/E_6 ratios were determined from the ratio of light absorption at 465 nm and 665 nm of a 1 mg sample dissolved in 10 mL of 0.05 mol L^{-1} $NaHCO_3$[6]. Total acidity (COOH) + (ϕ-OH) (mmolH^+/g OM); (OM: organic matter) and carboxyl groups (mmolH^+/gOM) were determined by the barium hydroxide and calcium acetate exchange methods, respectively. Phenolic hydroxyl groups (mmolH^+/gOM) were calculated as the difference between total and carboxylic acidities[6]. Acidity determination of the HA were also carried out under argon atmosphere to minimize absorption of carbon dioxide and air oxidation of the sample in an alkaline medium. C, H and N elemental analyses were performed on a Perkin Elmer model 240 analyzer, Mn, Fe, Co and Cu analyses, on a Varian model 250 PLUS atomic absorption spectrophotometer. For that porpose solutions obtained by HA samples treatment with concentrated HNO_3:HCl, 3:1, were conventionally introduced into an air-acetylene flame. Coefficients of variation between replicate metal analyses averaged 1%. Thermal analyses - differential scanning calorimetry (DSC) and thermogravimetry (TG) - were done on a simultaneous thermoanalyzer Netzsch (STA 409 Serie EP). The samples, typically 15 mg, were analyzed in a static atmosphere of air, heated at a rate of 5°C min^{-1} in the temperature interval of 30°C to 950°C. The samples were placed in alumina (Al_2O_3) crucibles, using empty crucibles as the reference. Enthalpy variation (ΔH) values were determined from the peak areas of the DSC curves, using the indium fusion enthalpy as the reference (T_f = 429,76 K; (H_f = 28,5 J g^{-1}).

Results and Discussion

Table 1 shows the analytical data of the HA samples studied in this work, and a average values of numerous analyses of purified HA extracted from soils from widely differing pedological origins[7]. The results showed % H and % C comparable or lower and %N higher than those normally reported for soil HA, and analogous to those recorded for some actinomycete and fungal melanins[8,9]. In the HA series, %N increases and %H and %C generally decrease in the order SH-HA, CO-HA, GO-HA, RA-HA. The values close to 1 obtained for the H/C atomic ratios are indicative of the strong aromatic and/or conjugated double bond character of these HA. In particular, the low H/C ratio found for RA-HA (0,82) suggests the presence of highly substituted (with non aliphatic substituents) or condensed aromatic structures. In the HA series, the N/C atomic ratios are higher than in soil HA (N/C < 0,06). In GO-HA and RA-HA the N/C values are close to those found in sewage sludge HA (N/C = 0.13)[10]. Based on the H/C and O/C ratio values, the

Table 1. Elemental composition, atomic ratios, ash contents and visible spectroscopic data of the HA samples

Sample	**%C**[a]	**%H**[a]	**%N**[a]	**%O+S**[b]	**H/C**[c]	**N/C**[c]	**O/C**[c]	**% Ash**[d]	**E_4/E_6**[e]
SH-HA	60.22	4.77	4.44	30.55	0.95	0.06	0.43	23.67	1.67
CO-HA	53.81	4.10	5.20	36.89	0.92	0.08	0.52	22.63	2.81
GO-HA	48.14	4.45	5.84	41.57	1.11	0.10	0.58	14.87	1.31
RA-HA	48.35	3.30	7.80	40.55	0.82	0.14	0.57	18.60	1.88
SO-HA[f]	56.2	4.8	3.2	36.3	1.0	0.05	0.5	—-	4.8

[a] calculated on dry ash free matter basis; [b] calculated by difference; [c] atomic ratios; [d] calculated on dry matter basis; [e] electronic spectrophotometric absorption ratio at 465 and 665 nm; [f] average values of several soil HA[7].

similarity of these HA with sediments of type III can be established, corresponding to recently formed humic substances, with a predominance of aromatic structures[11].

All of the HA samples studied presented metal concentrations in the following order: Fe > Mn > Cu > Co (Table 2). RA-HA presented the highest Cu content, whereas the highest Fe content was found in CO-HA. This latter sample also presents the lowest Mn content. Co is highest in SH-HA and lowest in GO-HA. Mn, Cu and Co concentrations were within the normal range for this metal in soils[12]. The speciation of the metal ions in these HA samples will be studied using electronic paramagnetic resonance (EPR) spectroscopy, in the part II of this work.

The enhanced ash contents in SH-HA and CO-HA (Table 1), among the HA samples studied, is noteworthy. This could be attributed to diet, because cows, for instance, eat the grass along with the roots, and so earth is gulped at the same time[13].

Table 2. Total quantity ($\mu g\ g^{-1}$ HA) of manganese (Mn), iron (Fe), cobalt (Co) and copper (Cu) in the HA samples.

Samples	Mn	Fe	Co	Cu
SH-HA-	700	2800	10	66
CO-HA	400	14400	9	153
GO-HA	1800	2900	3	62
RA-HA	700	4300	7	226
SOIL[a]	20-10000	——	0.5-65	2-250

[a] normal range for metals in soils[12]

In general, the H/C atomic ratios and the E4/E6 electronic spectrophotometric ratio values for the samples are lower (Table 1), as compared to those of soil-HA[7]. This suggests a high degree of conjugation and/or condensation of aromatic constituents, but not necessarily a high molecular weight for those VC fractions[14].

A study of the HA samples by thermal analysis (DSC and TG) has been carried out (Figure 1, Table 3). Very few thermoanalytical data for VC and their HA have been

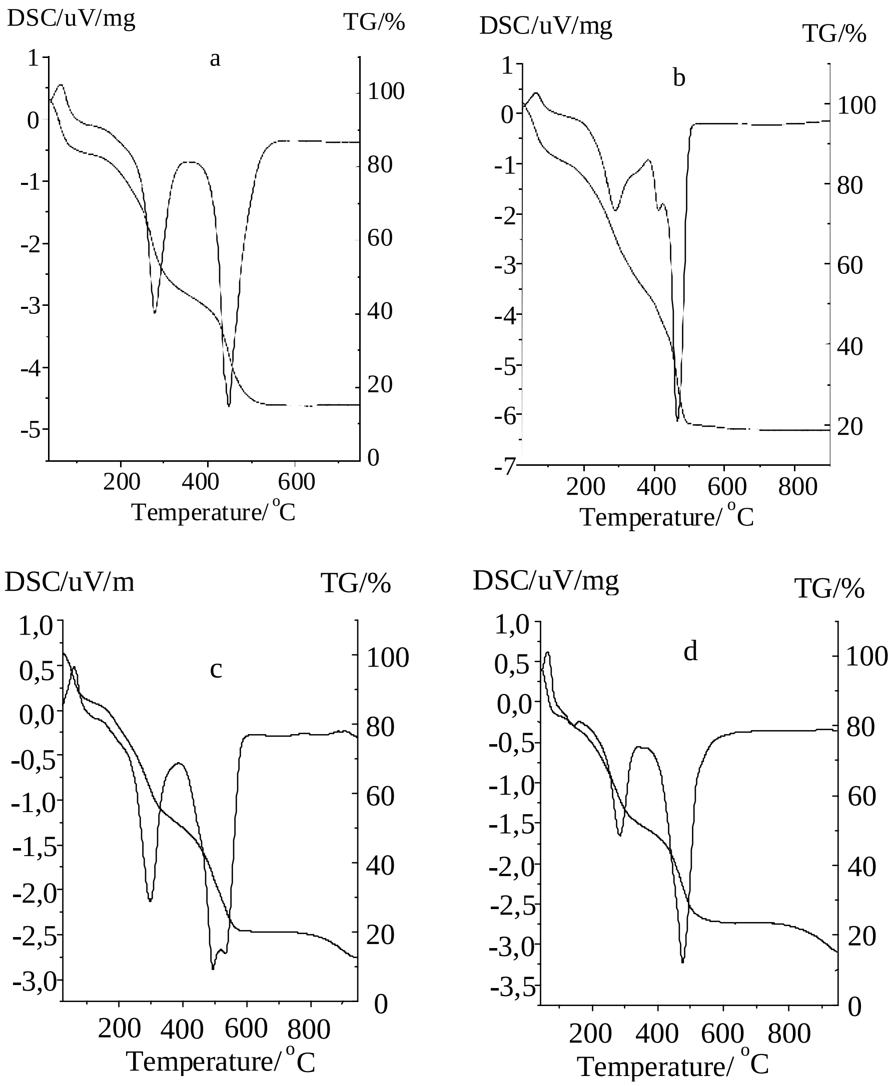

Figure 1. Thermal analysis curves (TG and DSC) 0f the humic acid samples (A=SH-HA; B=CO-HA; C=GO-HA; D=RA-HA.

Table 3. Thermal analysis data (DSC and TG) of the HA samples:

Samples	**Thermal Events**		**~300°C**	**~500°C**
SH-HA	temperature range (°C)		160 – 350	350 - 600
	ΔH (J/g)		-1574	-3034
	loss of mass (%)	a:	36,3	29,7
		b:	55,0	45,0
CO-HA	temperature range (°C)		160 – 384	384 - 600
	ΔH (J/g)		-1399	-3526
	loss of mass (%)	a:	32,0	33,0
		b:	49,2	50,8
GO-HA	temperature range (°C)		160 – 390	390 - 600
	ΔH (J/g)		-1209	-3270
	loss of mass (%)	a:	33,5	31,5
		b:	51,5	48,5
RA-HA	temperature range (°C)		160 – 350	350 - 600
	ΔH (J/g)		-892	-2516
	loss of mass (%)	a:	27,3	27,9
		b:	49,5	50,5

[a] in % on total samples basis; [b] in organic matter basis

reported, due probably to the low resolution of the curves[13]. However, extensive studies by pyrolysis-mass spectrometry of soil HA have been reported[15,16,17,18].

In this work HA DSC curves showed well-defined thermal events. DSC curves showed a short endothermic peak attributed to the moisture loss (t = 125 °C), and two strong exothermic peaks at ca. 300 °C and 500 °C, attributed to the decomposition of the organic matter (Figure 1). The DSC curves showed that the release of energy at ~ 500 °C was higher than at ~ 300 °C. TG curves indicated a continuous loss of mass which prevents a rigorous quantitative study. However, in order to observe the different tendencies of the HA samples on thermal analysis, we have carried out a semiquantitative study, that shows approximately the same loss of mass in both degradation processes, in terms of percentage of total sample or % of organic matter. The SH-HA sample presented a greater loss of mass at ~ 300 °C and at ~ 500 °C (Table 3). The calculated higher release of energy per unit mass of the processes at ~ 500 °C suggests the presence of a significant number of strong bonds in the four HA studied.

Humic substances seem to be constituted by alkylaromatic cores which can bind carbohydrates, proteins, lipids and inorganic substances[18,19,20]. The bimodal thermal profiles of the DSC curves are indicative of two types of bonds for the stabilization of organic matter in humic substances: intramolecular bonds between the components of the organic matter (peak at *ca*. 300 °C), and intermolecular organo-mineral linkages (peak at *ca*. 500 °C), the latter yielding more thermostable materials than the former[18,20].

On the basis of previous results and those reported here, we suggest that free inorganic matter (not bonded to the organic matter) and weakly bonded peripheral organic constituents were partially lost during the HA extraction processes from VC[21]. This fact is particularly enhanced in the cow samples, where a transformation of the original organic matter in the HA extraction process is suggested. The loss of carbohydrates, lipids and proteins, or its fragments, would be consistent with the higher aromatic content of the organic matter of HA, deduced above from their low H/C atomic ratio and E4/E6 values. However, the loss of protein contrasts with the high %N of these HA samples. This fact can be explained assuming that a large part of nitrogen can constitute heterocyclic structures of the HA cores[22,23], which is to be confirmed by ^{1}H-NMR and EPR spectroscopic analysis (part II of this work).

Total and carboxylic acidities of the HA samples have been determined (Table 4). In order to minimize distortions caused by the differences in ash contents of the samples, data are given on an organic matter basis. Thus, an error is made by considering the possible inorganic acidity as organic acidity.

Table 4. Total, carboxylic and phenolic acidities of the HA samples. For other abbreviations see text.

Acidities	SH-HA	CO-HA	GO-HA	RA-HA	SO-HA[c]
total ac.[a]	8.20	8.22	5.29	5.29	6.7
COOH ac. [a]	1.58	1.84	1.54	1.54	3.6
ϕ- OH ac.[a, b]	6.62	6.38	3.75	3.75	3.1

[a] in mmolH$^+$/g organic matter; [b] calculated by difference. [c] means of several soil HA[7].

Total and carboxylic acidities are analogous and lower respectively than those normally found in soil HA[7]. The low values of carboxylic acidity, characteristic of low humified materials, are comparable to those reported for some fungal and actinomycete melanins[8,9,22]. The low carboxylic acidity of these recent samples could be expected, since the oxidation of methyl groups, one of the sources of carboxylic acid groups[8,9] as the humification process advances seams be not accelerated by the earthworms work. In the HA series, GO-HA and RA-HA show the lowest carboxylic and phenolic contents, (Table 4). The phenolic acid character of GO-HA and RA-HA are the most similar to that of the soil HA, in accordance with the analytical data.

The infrared spectra of the HA samples (Figure 2) are consistent with the data obtained by chemical analysis. The presence of a band at 3690 cm^{-1} only in the CO-HA spectrum, which can be assigned to the (SiO-H), is consistent with the high ash content in this sample. All spectra show a very broad and strong absorption centered at 3426-3409 cm^{-1}, assigned to ν(O-H) of alcoholic or phenolic groups. The shoulder of these bands at 3261-3226 cm^{-1} can be attributed to ν(N-H) of amide groups or ν(O-H) of H-bonded phenolic groups. The pattern of the bands in the 1740-1600 cm^{-1} region is analogous to that found in the IR spectra of actinomycete and fungal melanin, characteristic of young HA[8,9]. Thus, all spectra show two partially overlapped bands at 1720 and 1645 cm^{-1} of moderate and strong intensity respectively, the former assigned to ν(O-C=O) of esters and carboxylic acids or ν(C=O) of ketones, and the latter attributed to ν(O-C=O) of metal-coordinated carboxylates, H-bonded ν(C=O) or ν(C=O) of amides (amide I band). The low intensity of the band at 1720 cm^{-1} is in agreement with the low carboxylic acidity of the samples determined by chemical analysis (Table 4).

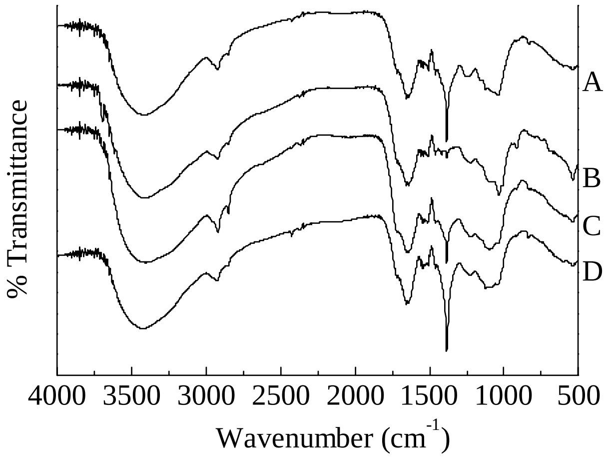

Figure 2. FTIR spectra of the humic acid samples (A=SH-HA; B=CO-HA; C=GO-HA; D=RA-HA).

The broad absorption centered at 1645cm^{-1} appears in all cases to be split in three components, indicating different vibrational types. Thus, an H-bonded ν(C=O) vibration would be consistent with the phenolic content of the samples. These phenol groups would be in the ortho position relative to the carboxylic aromatic substituent, yielding salicylic type moieties[26]. The presence of carboxylate complexes of metals, mainly of manganese, iron and copper, will be tested later by EPR spectroscopy (part II of this work), and the existence of amide groups in the samples, consistent with their high nitrogen content, agrees with the interpretation of the melanin IR spectra[8,9]. The absorption band at 1645 cm^{-1} also shows a shoulder at 1614 cm^{-1} in all spectra, which could be tentatively assigned to a ν(C=N) stretching band of aromatic heterocyclic groups. Due to the high aromatic content of the samples, the assignment of the amide II band is not trivial, because it appears in the same region as the ν(C=C) of aromatic compounds. Nevertheless, an absorption which appears in all cases at 1543-1540 cm^{-1} can be tentatively attributed to this vibration. With exception of CO-HA, the IR spectra of the HA samples are dominated by a very sharp absorption at 1384 cm^{-1}, unusual in humic substances, that has been tentatively assigned to aliphatic ν(CH_2). The CO-HA spectrum shows weak bands in this region which can be attributed to ν(CH_2), phenolic ν(C-O) or ν_{sym}(OCO) of coordinated carboxylate groups.

The existence of carbohydrates in the samples is suggested[10] by one band at 1036-1028 cm^{-1}, although the assignment is not unequivocal due to the broad ν(Si-O) which appears in the same region. The IR spectra are consistent with the phenolic content, the low carboxylic acidity and the high %N of the samples. The nitrogen seems to constitute secondary amide groups and aromatic heterocycles.

Conclusions

From the combined data of chemical, thermal and spectroscopic analysis, we can conclude that the HA samples studied here show a high degree of conjugated and/or substituted or condensed aromatic structure, as well as a low carboxylic acidity content. These facts suggest, in these recent HA from VC, a high degree of internal humification by aromatic condensation, and a paradoxical low degree of peripheral humification, because they lack carboxylic acid groups, the oxidation products of aromatic methyl substituents.

The high nitrogen content of the samples agrees with the presence of bands in the IR spectra corresponding to secondary amide groups, suggesting existence of peptidic chains in the samples from protein degradation processes. The presence of nitrogen heterocycles also suggested.

On the basis of the study carried out, a similarity between these HA samples and soil HA can be established in the order SH-HA > CO-HA > GO-HA >RA-HA. Thus, RA-HA would be the least similar to soil HA due to their low H/C and high N/C atomic ratios, their low carboxylic acid character and high degree of aromaticity.

As the treatment with *Eisenia foetida* of the respective manures has been the same in the four cases, the difference in the properties of the different HA, and of the VC where they were extracted, could be due to different nourishment of the animals or to different processes in their digestive systems. Our results show that it is possible to prepare specific worm-composts to solve specific problems of degraded soils.

With the aim of confirming and extending knowledge of the chemical characteristics of these HA and WC material, we are studying their nuclear magnetic and electronic paramagnetic resonance spectra, which will constitute part II of this work.

Acknowledgements

This work was supported by CNPq fellowships and by grants from CNPq and PADCT/FINEP. The authors are also grateful to Prof. Dr. I. M. Brinn for assistance with the English text and for useful comments on the manuscript.

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Received: May 17, 1999